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Abstract: In species with biparental care, a female gains fitness benefits from the joint reproductive investment of herself and her partner, but pays only the costs of her own care. Selection thus favours mechanisms that allow females to elicit a higher paternal investment from their partners. In oviparous species, the allocation of maternal yolk androgens to the eggs might represent such a female adaptation to sexually antagonistic selection. To test this hypothesis, we experimentally blocked the effects of maternal yolk androgens by an injection of the antiandrogen flutamide or a control substance in the eggs of great tits, Parus major. We subsequently manipulated the food demand of the brood in a brood size manipulation experiment, and recorded the parental feeding rates. The males' food-provisioning rates were not significantly influenced by the actions of maternal yolk androgens, whereas females adjusted their parental effort to androgen-mediated nestling signals, in particular in enlarged broods. These results show that female great tits do not exploit the male's contribution to parental care by allocating high concentrations of yolk androgens to their eggs. However, they indicate that variation in yolk androgen allocation among females has evolved through a process of coadaptation that matches maternal food provisioning and offspring demand.

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Differential effects of yolk hormones on maternal and paternal contribution to parental care

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In species with biparental care, a female gains fitness benefits from the joint reproductive investment of herself and her partner, but pays only the costs of her own care. Selection thus favours mechanisms that allow females to elicit a higher paternal investment from her partner. In oviparous species, the allocation of maternal yolk androgens into the eggs might represent such a female adaptation to sexually antagonistic selection. To test this hypothesis, we experimentally blocked the effects of maternal yolk androgens by an injection of the anti–androgen flutamide or a control substance in the eggs of great tits (*Parus major)*. After hatching, we subsequently manipulated the food demand of the brood in a brood size manipulation experiment, and recorded the parental feeding rates. We found that the males' food provisioning rates were not significantly influenced by the actions of maternal yolk androgens, while females adjusted their parental effort to androgen-mediated nestling signals, in particular in enlarged broods. These results show that female great tits do not exploit the male's contribution to parental care by allocating high concentrations of yolk androgens into their eggs. It however indicates, that variation in yolk androgen allocation among females has evolved through a process of coadaptation that matches maternal food provisioning and offspring demand.

Keywords: coadaptation, food provisioning, great tit, maternal effects, maternal yolk androgens, parental investment, parent-offspring communication, *Parus major,* sexual conflict

In sexually reproducing species, a conflict over parental investment occurs because each parent gains fitness benefits from the joint reproductive investment of both partners, but pays only the costs of its own care. Selection will thus favour adaptations that successfully manipulate the genetically unrelated partner into elevating its parental investment, which, as a consequence, will allow the manipulator to reduce its own reproductive contribution (Trivers 1972; Houston & Davies 1985; Lessells 1999; McNamara et al. 1999; Arnqvist & Rowe 2005; Houston et al. 2005).

In blue tits (*Cyanistes caeruleus*), for example, a sexual conflict over the degree of hatching asynchrony occurs (Slagsvold et al. 1994, 1995). Females pay relatively lower costs of parental care in terms of future survival when raising even-aged nestlings, while males survive better when caring for an asynchronous brood (Slagsvold et al. 1994, 1995). While the total cost of parental care is similar for synchronous and asynchronous broods, the different hatching patterns thus alter the relative reproductive burden on the two sexes (Slagsvold et al. 1994, 1995). Because only the female incubates in this species, she can control the degree of hatching asynchrony by varying the start of incubation, and is thus likely to win this conflict (Slagsvold et al. 1994, 1995).

Not only the start of incubation, but any reproductive behaviour that is under female control alone, has the potential to mediate sexual conflicts over reproductive decisions in favour of the female's own interest. In birds (Schwabl 1993; Groothuis et al. 2005), fish (McCormick 1999), and reptiles (Bowden et al. 2000; Lovern & Wade 2003), for example, females are known to deposit androgens and other hormones into their eggs. The amount of yolk

74 hormones varies however greatly, not only among species, but also within and
75 among clutches of the same species and even among clutches of the same
76 female. Variation within clutches, i.e. increasing or decreasing hormone
77 concentrations with laying order, might aim at mitigating the competitive
78 asynchrony among siblings caused by asynchronous hatching (e.g. Eising et
79 al. 2001; Pilz et al. 2003) or enhance these effects to facilitate brood reduction
80 when food is limited (Schwabl et al. 1997). However, although variation in the
81 amount of yolk hormones deposited *among* clutches of different females is
82 typically even larger (e.g. Reed & Vleck 2001; Groothuis & Schwabl 2002; Pilz
83 et al. 2003; Tschirren et al. 2004), the mechanisms driving this variation are
84 as yet poorly understood (Groothuis et al. 2005, Müller et al. 2007b).
85 Experimental studies on a number of bird species have shown that
86 yolk androgens deposited by the mother can have profound effects on the
87 development, morphology and food acquisition behaviour of nestlings
88 (reviewed in Groothuis et al. 2005). Young birds originating from eggs with
89 experimentally high yolk androgens concentrations showed, for example, a
90 higher begging effort (Schwabl, 1996; Eising & Groothuis 2003; von
91 Engelhardt et al. 2006; but see Pilz et al. 2004; Boncoraglio et al. 2006) and a
92 higher growth rate (Schwabl, 1996; Eising et al. 2001; Tschirren et al. 2005)
93 than their unmanipulated siblings. Further, maternal yolk androgens can
94 promote the development of the *musculus complexus*, a neck muscle involved
95 in food acquisition (Lipar & Ketterson 2000), and beak flange size, which
96 might stimulate parental food provisioning (Müller et al. 2007b). Yolk
97 hormones can thus influence a variety of physiological, behavioural and

98 morphological nestling traits, all of which may affect parental feeding
99 decisions.
100 If males are especially sensitive to such hormone-dependent nestling
101 signals, then the deposition of yolk androgens by the female could represent a
102 sexually antagonistic adaptation that aims at exploiting the male's contribution
103 to parental care, as recently suggested by Michl et al. (2005), Moreno-Rueda
104 (2007) and Müller et al. (2007a). To test this, we experimentally inhibited the
105 effects of maternal yolk androgens by injecting flutamide, an androgen
106 receptor inhibitor, or a control substance into the eggs of free-living great tits
107 (*Parus major*), and filmed the food provisioning behaviour of the parents to
108 measure their investment.
109 Flutamide directly competes with testosterone and testosterone-
110 metabolites for binding to androgen receptors (Simard et al. 1986), and
111 previous work on chicken (*Gallus gallus*; Burke 1996), red-winged blackbirds
112 (Lipar & Ketterson 2000) and black-headed gulls (Müller et al. 2005) has
113 confirmed its anti-androgenic actions when injected *in ovo*. If the deposition of
114 yolk androgens into the eggs is a sexually antagonistic adaptation of the
115 female to increase the male's contribution to parental care, we predict 1)
116 higher feeding rates of the male in control-injected compared to flutamide-
117 injected (i.e. androgen-blocked) broods, and 2) that the effect of yolk
118 androgens on the parental food provisioning behaviour will be most
119 pronounced when the nestlings' degree of hunger, and thereby the cost of
120 rearing the brood, is increased (i.e. in experimentally enlarged broods).
121
122

METHODS

Study Site, Species and Flutamide Injection

The experiment was performed in 2003 in a great tit population (*Parus major*) breeding in nest boxes in a forest near Bern, Switzerland ("Forst", 46°54'N 7°17'E / 46°57'N 7°21'E). Great tits are socially monogamous passerines that rear one, or in a few cases two broods per year. Only females build the nest and incubate the eggs but both parents feed the young during the nestling stage (see e.g. Tschirren et al. 2005a for a typical division of work between the sexes).

All nest boxes were cleaned before the start of the breeding season (February) to remove nest-based ectoparasites, which can influence yolk androgen deposition (Tschirren et al. 2004). From the beginning of the breeding season (April) onwards, we regularly visited nest boxes to determine the start of nest building and egg laying. After clutch completion, we injected all eggs of a clutch with either 0.0319μmol of the anti-androgen Flutamide (Fluka, Switzerland) dissolved in 5μl ethanol (70%) or with 5μl ethanol (70%) as a control (see Lipar & Ketterson 2000 for details). The injected dose of flutamide was based on the amounts used in Burke (1996) and Lipar & Ketterson (2000), adjusted for an average yolk mass of 352mg in our study population. The injections were done in the field using a 25μl syringe (Hamilton 702LT) and a 25 G needle (see Tschirren et al. 2005b for details). During the injection procedure, the eggs were illuminated from beneath using a cold light source (Intralux 4000, Volpi, Switzerland) to ensure that the tip of the needle penetrated the yolk membrane. The hole in the eggshell was sealed by applying a small drop of tissue adhesive (Vetseal, B. Braun

148 Medical, Switzerland). The average hatching success of the eggs was 77.2%.
149 It did not significantly differ between flutamide-injected and control eggs (χ^2=
150 0.45, *P*= 0.502, N=534).
151

152 **Brood Size Manipulation**

153 One day after hatching, we created broods of reduced (-2 nestlings compared
154 to original clutch size) and enlarged size (+ 2 nestlings compared to original
155 clutch size) by partially exchanging randomly chosen nestlings among nests
156 with the same flutamide treatment, the same hatching date and a similar
157 clutch size. After the manipulation, both enlarged and reduced nests
158 contained own and foster nestlings of the same flutamide-treatment group,
159 and the manipulated brood sizes remained within the range of natural
160 variation observed in our study population (5–12 nestlings / brood). This
161 manipulation allowed us to assess the investment of the parents in response
162 to an elevated or reduced food demand of the brood, and the interaction
163 effects between the food demand of the brood and the effects of maternal yolk
164 androgens (i.e. a 2x2 design).
165 The original clutch size did not differ significantly between treatment
166 groups (brood size manipulation, $F_{1, 46}$ = 0.01, *P* = 0.941; flutamide treatment,
167 $F_{1, 46}$ = 0.81, p= 0.373; interaction, $F_{1, 45}$ = 0.07, *P* = 0.800). After the
168 manipulation, the brood size was significantly larger in enlarged compared to
169 reduced broods (brood size manipulation, $F_{1, 46}$ = 187.24, *P* < 0.001; R^2=
170 0.80), but did not differ significantly between the flutamide-treatment groups
171 ($F_{1, 46}$ = 0.42, *P* = 0.520; interaction, $F_{1, 45}$ = 0.08, *P* = 0.776).
172

Parental Food Provisioning

Ten days post-hatching, when the nestlings' food demand is highest (Gebhardt-Henrich 1990; Keller & Van Noordwijk 1994), we installed a video camera sensitive to infrared and equipped with an infrared lamp in the nest box to assess the parental investment in response to the flutamide treatment and the brood size manipulation. Video recording started 30 minutes after camera installation and the food provisioning behaviour of the parents was filmed during the following 100 minutes. After the filming, nestlings were weighted and ringed with individually numbered aluminium rings.

A total of 49 broods (11 enlarged, control-injected broods, 14 reduced, control-injected broods, 11 enlarged, flutamide-injected broods, and 13 reduced, flutamide-injected broods) were filmed. The analysis of the recordings was performed blindly with respect to the treatment of the nest. Males and females can be visually identified on the videos. Only visits during which the male or female parent delivered food to at least one nestling were counted as a feeding visit. In addition to the number of parental feeding visits, we also classified the prey size brought to the nestlings as small, intermediate or large (Kölliker et al. 1998). The food quantity delivered to the nestlings was then calculated as the product of feedings per hour and mean prey size. Because the analyses of feeding visits and food quantity delivered to the brood were qualitatively very similar (see also Moreno et al. 1995; Neuenschwander et al. 2003) only the results of the former are presented here.

The disturbance of the nest during the installation of the camera (performed within < 5 minutes) and the filming within the nest boxes (method

established in previous years; see e.g. Tschirren et al. 2005a) did not have a negative effect on the feeding behaviour of the parents. Both parents fed the nestlings on a regular basis during the filming in all experimental nests, and no nest desertion or nestling mortality was observed during the filming. The video recording or the disturbance of the nests during the manipulations did not have any observable adverse short- or long-term effects on the nestlings, and their weight (mean: 15.77 ± 0.10g) was similar to the weight of great tit nestlings in our study population in previous years (range of mean weights between 1997 and 2002: 14.49g–16.21g, depending on food availability).

Statistical Analyses

The flutamide treatment and the brood size manipulation influenced the feeding behaviour of males and females differently (see Results). We thus analysed the feeding behaviour of mothers and fathers separately. Maternal and paternal feeding visits were analysed with ANCOVAs including the flutamide treatment, the brood size manipulation, and their interaction as fixed effects and nestling body mass at filming as a covariate. The interaction was removed from the final model if non-significant. For the analysis of differences between flutamide-injected and control-injected broods within a given brood size manipulation group we used least-square mean contrasts.

All tests were two-tailed with a significance level of $P \leq 0.05$. Residuals of the models were tested for normality using Shapiro-Wilk tests and homoscedasticity using Bartlett tests to ensure that the assumptions for parametric testing were fulfilled. Means ± 1 S.E. are presented in the results

and figures. Statistical analyses were performed using JMP IN 5.1 (Sall & Lehmann 1996).

RESULTS

The flutamide treatment and the brood size manipulation influenced the feeding behaviour of the two parents differently (three-way interaction: parental sex x brood size manipulation x flutamide treatment, $F_{1, 44}= 6.08$, $P= 0.018$). We therefore analysed the feeding behaviour of mothers and fathers separately.

Paternal Food Provisioning

Contrary to our prediction, we found no significant effect of the flutamide treatment ($F_{1, 45} = 0.17$, $P = 0.685$) and no significant interaction effect of the flutamide treatment and the brood size manipulation ($F_{1, 44} < 0.01$, $P = 0.993$) on the males' feeding rates (Fig. 1a). Independent of the flutamide treatment of the young, males raising an enlarged brood fed at significantly higher rates than males raising a reduced brood ($F_{1, 45} = 4.87$, $P = 0.033$, Fig. 1a). Male feeding rates were not significantly influenced by nestling body mass at filming ($F_{1, 45}= 2.61$, $P= 0.114$). Further, no effect of the brood size manipulation on the males' feeding visits *per nestling* were found ($F_{1, 45} = 1.95$, $P = 0.170$), showing that males increased their feeding effort in enlarged broods to keep the food provisioning per nestling constant. Male feeding rates were not significantly influenced by the date of filming ($F_{1, 41}= 2.19$, $P= 0.147$), the time of filming ($F_{1, 41}< 0.01$, $P= 0.932$), the original clutch size ($F_{1, 41}= 0.31$, $P=0.578$), or the partner's feeding rate ($F_{1, 41}= 0.174$, $P=0.679$).

247

248 **Maternal Food Provisioning**

249 We found a significant interaction effect of the flutamide treatment and the
250 brood size manipulation on the mother's feeding visits ($F_{1, 44}$ = 8.65, P =
251 0.005, Fig. 1b). When the food demand of the brood was low (i.e. in reduced
252 broods), mothers did not feed control-injected and flutamide-injected nestlings
253 at significantly different rates (contrast: $F_{1, 44}$= 0.04, P = 0.851), whereas when
254 the food demand of the brood was high (i.e. in enlarged broods), mothers of
255 control-injected broods showed significantly higher feeding rates compared to
256 mothers of flutamide-injected broods (contrast: $F_{1, 44}$= 17.15, P <0.001).
257 Similarly, within the control-injected group, mothers feeding an enlarged brood
258 showed significantly higher feeding rates than mothers feeding a reduced
259 brood (contrast $F_{1, 44}$= 21.50, P <0.001), while in the flutamide-injected group
260 the difference in the females' food provisioning behaviour between enlarged
261 and reduced broods was not statistically significant (contrast $F_{1, 44}$= 0.46, P
262 =0.499). Thus, females did not adjust their food provisioning to the higher food
263 demand of enlarged broods when the actions of maternal yolk hormones were
264 blocked. Similar results were found when analysing the females' feeding visits
265 per nestling instead of the total feeding visits per brood (interaction brood size
266 manipulation x hormone treatment: $F_{1, 44}$= 4.30, P =0.044), indicating that
267 females overcompensated in response to the brood size manipulation.

268 Female feeding rates significantly increased during the breeding
269 season ($F_{1, 43}$= 4.27, P =0.045) and tended to decrease with increasing
270 nestling body mass ($F_{1, 43}$= 3.03, P =0.089). However, including these
271 covariates into the analysis did not change the interaction effect between the

brood size manipulation and the hormone treatment on female feeding rates (interaction: $F_{1, 43}$= 10.28, P= 0.003). Female feeding rates were not significantly influenced by the time of video recording ($F_{1, 40}$= 0.13, P= 0.723), the original clutch size ($F_{1, 40}$= 0.17, P=0.681), or the partner's feeding rate ($F_{1, 40}$= 0.13, P=0.718).

DISCUSSION

In this study we experimentally investigated if maternal yolk androgens represent a mechanism by which females can manipulate their partner's investment in parental care, as recently suggested by Michl et al. (2005), Moreno-Rueda (2007) and Müller et al. (2007a). Counter to the predictions of this hypothesis, we found that males adjusted their feeding effort solely to the number of nestlings present in the nest, irrespective of the flutamide-treatment of the young. Likewise, in control-injected broods, females increased their feeding visits when caring for an enlarged brood. However, when the actions of maternal yolk androgens were experimentally inhibited, (i.e. in the flutamide-injected group), an increased food demand of the brood did not lead to a higher maternal investment. Thereby these results indicate that yolk androgens allow nestlings to elicit higher feeding rates from their mother, especially when their food demand is increased (i.e. when environmental conditions get unfavourable; see also Pilz et al. 2004).

Our finding that yolk hormones have an effect on maternal, but not on paternal investment, suggests that selection may have reduced the males' susceptibility to yolk androgen-mediated nestling signals to resist the exploitation of their parental effort by the female (see also Müller et al. 2007a).

297 Indeed, evidence that males ignore certain nestling displays has been found
298 in canaries, where males adjust their food provisioning to the chicks' begging
299 posture only, while females respond to a variety of offspring signals (Kilner
300 2002b). Similarly, male great tits adjust their feeding behaviour to visual
301 displays, while females integrate visual as well as vocal nestling signals
302 (Kilner 2002a).

303 While males were not susceptible to the effects of yolk androgens,
304 females did adjust their feeding behaviour in response to the flutamide
305 treatment, especially when feeding an enlarged brood. This shows that
306 maternal yolk hormones play an important role in regulating mother-offspring
307 interactions. Indeed, Kölliker et al. (2000) showed by means of a partial cross-
308 fostering experiment, that the level of food solicitation by the nestlings is
309 largely dependent on their nest of origin, and that the female's increase in
310 food provisioning in response to an increased demand was positively
311 correlated to the natural levels of begging intensity of her offspring. No such
312 correlation was found in the paternal line. Although the role of (additive)
313 genetic variation cannot be excluded (Kölliker et al. 2000), our finding
314 supports the idea that this correlation between offspring demand and maternal
315 response may at least partly be mediated or enhanced by maternal effects
316 (see also Kölliker et al. 2000; Kölliker et al. 2005).

317 The goal of this study was to investigate the effects of yolk hormones
318 on parental feeding investment, which does not require a direct measurement
319 of the numerous potential pathways that could mediate such effects. However,
320 now we know that yolk hormones do indeed affect maternal investment, and
321 that this effect is independent of nestling body mass, elucidating these

322 proximate mechanisms is an obvious next step. In particular, to test whether
323 males specifically ignore yolk androgen-dependent nestling signals to avoid
324 exploitation of parental care by the female, we will need to experimentally
325 investigate the effects of yolk hormones on as many different aspects of
326 offspring behaviour and morphology as possible, and to test for sex-specific
327 sensitivities to these traits.

328 In conclusion, our study shows that female great tits do not exploit the
329 male's contribution to parental care by differentially allocating yolk androgens
330 into the eggs. However, the females' own adjustment of parental effort in
331 response to yolk androgen-mediated nestling signals indicates that maternal
332 hormones do play an important role in mother-offspring communication, and
333 that variation in the deposition of yolk androgens between females may have
334 evolved to optimally match maternal supply and offspring demand, as
335 predicted by Kölliker et al. (2005). This maternal sensitivity to the actions of
336 yolk hormones might ensure a prime start for offspring, and particularly for
337 those of high reproductive value (see e.g. Gil et al. 1999, 2004; Tanvez et al.
338 2004; Loyau et al. 2007), even if environmental conditions get unfavourable.
339 Interestingly, the mother's sensitivity to her offspring's need also makes her
340 vulnerable to exploitation by her nestlings (Trivers 1974; Godfray 1995).
341 However, our finding that females did, unlike males, maintain their sensitivity
342 to yolk androgen-mediated nestling signals suggests that the costs of ignoring
343 such signals are sex-specific, and that in females they do not exceed the
344 benefits. Although more research is necessary to gain a full understanding of
345 the adaptive value of variation in yolk hormone deposition among females,
346 and the covariance between provisioning and soliciting behaviour caused by

347 genetic variation in maternal hormone levels, our study indicates that maternal
348 yolk hormones can play an important role in shaping the coadaptation among
349 mothers and their young.
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351
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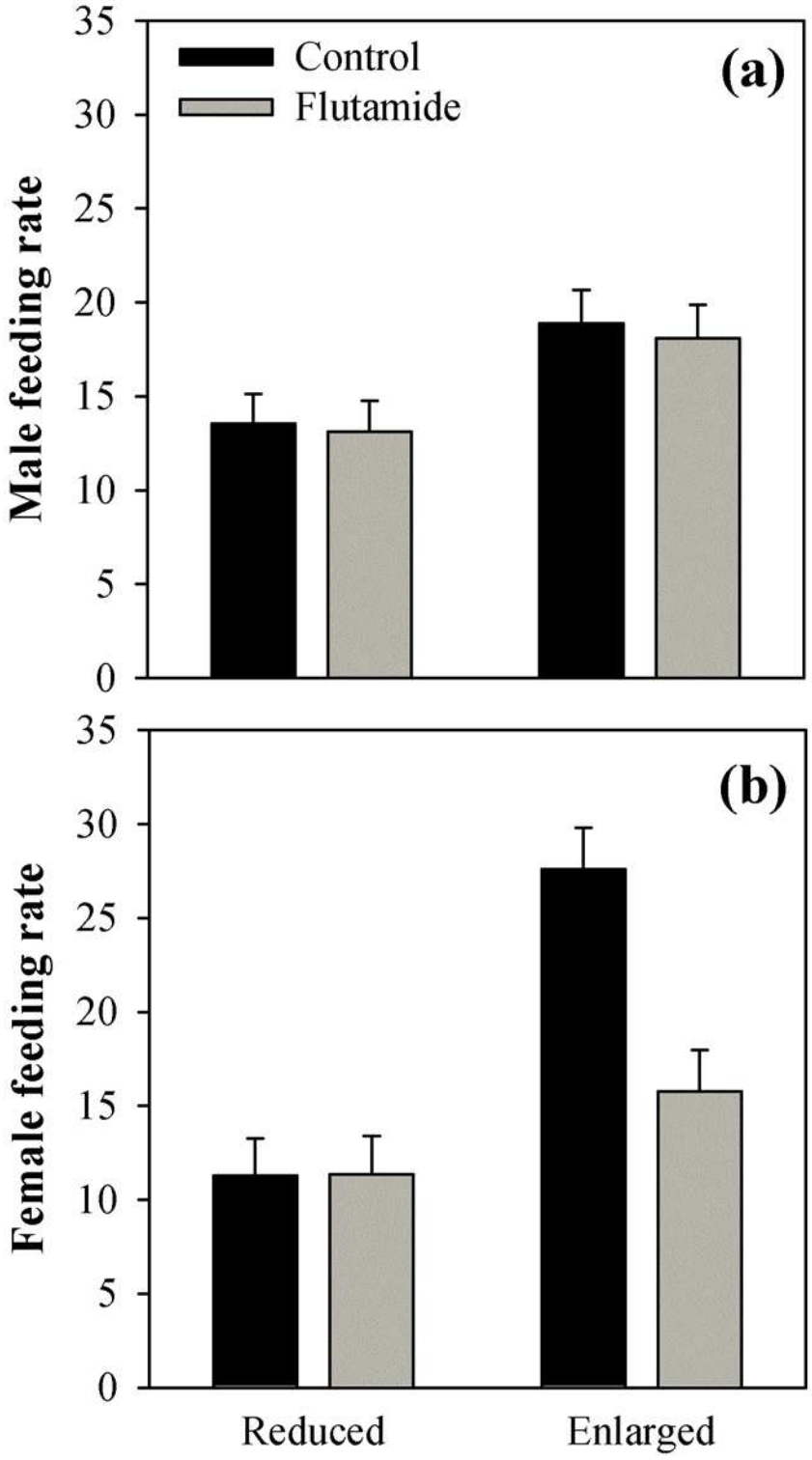
FIGURE LEGEND

Figure 1

Feeding visits per hour of a) male, and b) female great tits in relation to the flutamide treatment of the nestlings (nestlings originating from flutamide- or control-injected eggs) and the manipulated brood size (feeding an enlarged or reduced brood). Least square means + 1SE are shown.

520 **FIGURE**



521

522 **Fig. 1**